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Genetic linkage in soybean : Linkage Group 8

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Genetic maps integrating conventional and molecular marker loci are valuable to plant breeders and geneticists. Linkage values are used to calculate progeny sizes necessary to obtain recombination between linked loci. Linked marker genes are used as screening aids in cultivar development programs. Detailed genetic maps are helpful for cloning genes to use in cultivar development or to investigate gene function.

Construction of genetic maps involves two parts: defining linkage groups, and mapping linkage groups to chromosomes. Both use segregation ratio analysis of test cross, backcross, F2, and subsequent generations to obtain information. Additional information is obtained using translocations, inversions, aneuploids, and restriction fragment length polymorphisms (RFLPs).

Conventional genetic marker maps and RFLP maps have been constructed for only a few economically important crops. Construction of a genetic map for soybean, Glycine max (L.) Merr., has proceeded slowly compared to some other crops. Progress has been limited by several factors. Soybean's economic value has been more recently realized. Cross pollination of soybean is difficult, time-consuming, and produces few seeds per pod. Soybean chromosomes are numerous, small, and difficult morphologically to distinguish from each other. Conventional marker mutant alleles usually are found alone in mutant lines making it difficult to obtain populations segregating for a large number of markers. However, progress is being made. Isozyme analysis of cultivars and mutant lines has increased the

number of loci available for segregation analysis and for incorporation into conventional marker maps. Separate RFLP maps also are being constructed. The conventional marker maps are being consolidated with RFLP maps into a single map using RFLP analysis of near isogenic lines (Muehlbauer et al., 1988) and other methods.

Continuing linkage studies in soybean are required to define the expected 20 linkage groups. Based on chromosome number, 20 linkage groups are expected in soybean. Linkage studies based on RFLPs have defined 23 linkage groups (Shoemaker et al., in press). Linkage studies using conventional markers have defined 20 linkage groups (Palmer and Kiang, 1990; Muehlbauer et al., 1989; Devine et al., 1991; Palmer et al., 1992). These may not necessarily correspond with 20 different chromosomes since only two linkage groups have more than four markers. Some of the linkage groups may be on the same chromosome, and some chromosomes may not be represented by a linkage group. Only one linkage group has been assigned to a chromosome.

Linkage Group 8 has been assigned to the satellite or nucleolus organizing chromosome (Sadanaga and Grindeland, 1984) and is one of the better described linkage groups. Linkage Group 8 is defined by seven known gene loci: W1, Wm, Ms1, St5, Adh1, Ms6, and Y23.

The W1 locus affects pigment production. Flowers and seedling hypocotyls of wild-type plants are purple-pigmented. Plants homozygous for the recessive allele at the W1 locus, w1 w1, are white-flowered and have green seedling hypocotyls lacking purple pigment. Many cultivars are w1 w1 white-flowered.

The Wm locus also affects pigment production. Plants homozygous for the recessive allele at the Wm locus, wm wm, are magenta-flowered. These mutants also have a lower photosynthetic rate, a reddish-purple discoloration of the leaves as the plants approach maturity, earlier leaf senescence, and lower yield (Buzzell and Buttery,1977). This mutant is maintained in the Genetic Type collection as T235.

The Ms1 locus affects fertility. Plants homozygous for the recessive allele at the Ms1 locus, ms1 ms1, are male sterile, female fertile. Sterility is caused by failure of cytokinesis after telophase II. Pollen stained with an iodine potassium iodide (I2KI) solution and examined with a light microscope is large and dark-staining (Albertsen and Palmer, 1979). When examined with crossing glasses, fresh anthers of ms1 ms1 plants are larger, brighter yellow, and shed little or no pollen. At maturity, ms1 ms1 plants are green with several fleshy seedless pods plus some seeded pods from outcrossing. Seven independent mutations have been recorded at this locus (Skorupska and Palmer, 1990). The Urbana male sterile, ms1 ms1, has a higher female fertility than the others. This mutant is maintained as the heterozygote in the Genetic Type collection as T266H.

The St5 allele also affects fertility. Plants homozygous for the recessive allele at the St5 locus, st5 st5, are male sterile and female sterile. The st5 mutant is a desynaptic mutant; there is almost complete absence of chromosome pairing at diakinesis (Palmer and Kaul, 1983). Pollen grains stained with an I2KI solution and examined with a light microscope are extremely variable in size. Less than 1% is well stained, and the centers appear shrunken away from the edges (Palmer and Kaul,

1983). When examined with crossing glasses, fresh anthers of st5 st5 plants appear small and grey-yellow, and shed little or no pollen. At maturity, st5 st5 plants are green with several fleshy pods. Seeded pods are rare; Palmer and Kaul (1983) counted only three seeds on 1000 sterile plants. This mutant is maintained as the heterozygote in the Genetic Type collection as T272H.

The Adh1 locus affects alcohol dehydrogenase (ADH, EC 1.2.3.4) production. When plants homozygous for the recessive allele at the Adh1 locus, adh1 adh1, are tested electrophoretically, a banding pattern is produced with band 1 absent (Gorman and Kiang, 1977; Gorman and Kiang, 1978; Appendix A). The adh1 allele is sometimes referred to as a "null" allele. Many cultivars are adh1 adh1 (Gorman et al., 1982).

The Ms6 locus affects fertility. Plants homozygous for the recessive allele at the Ms6 locus, ms6 ms6, are male sterile, female fertile. Sterility is due to tapetal abnormalities; anthers appear shrunken and devoid of pollen (Skorupska and Palmer, 1989). A pleiotropic effect is small flower size (Skorupska and Palmer, 1989). When examined with crossing glasses, fresh anthers of ms6 ms6 plants are translucent and red-brown; the color intensifies with age. At maturity, ms6 ms6 plants are green with many fleshy seedless pods plus several seeded pods from outcrossing. This mutant is maintained as the heterozygote in the Genetic Type collection as T295H.

The Y23 locus affects chlorophyll production. Plant homozygous for the recessive allele at the Y23 locus, y23 y23, have chlorophyll-deficient foliage. New leaves change in color from green to yellow-white and

eventually become necrotic. Many leaves abscise; plants are weak but viable (Palmer et al., 1990). This mutant is maintained in the Genetic Type collection as T288.

Linkage estimates for loci of Linkage Group 8 have come from several sources. Palmer (1976) calculated linkage of the Ms1 locus to the W1 locus with percentage recombination of 29.7% ± 1.2%, and to the breakpoint of chromosome interchange from PI 101,404B with percentage recombination 8.5% ± 1.5%. Linkage was not established between the W1 locus and the interchange breakpoint. Gene order was stated as: interchange breakpoint - Ms1 - W1.

Buzzell and Buttery (1977) showed linkage of the Wm locus to the W1 locus with percentage recombination of 2.2% ± 0.7%. Palmer and Kaul (1983) showed linkage of the St5 locus to the W1 locus with percentage recombination of 26.7% ± 1.9%. They also stated that previous data showed recombination between the Ms1 locus and the Wm locus of 30.1% ± 0.9%, but did not cite published data. Gene order was proposed as translocation breakpoint - Ms1 - W1- Wm - St5 (Palmer and Kaul, 1983). Palmer (1985)(no data given) illustrated gene order as Ms1 - Wm - W1 - St5.

Kiang and Chiang (1987) showed linkage of the Adh1 locus with the W1 locus with percentage recombination of 20.6% ± 1.2%. Skorupska and Palmer (1989) showed linkage of the Ms6 locus to the W1 locus with percentage recombination of 3.08% ± 0.1%. Kiang (1990) presented data showing several linkages: Adh1 to Wm with 28% ± 2% recombination; Adh1 to Ms1 with 42.1% ± 3% recombination; Adh1 to W1 with 24.9% ± 0.9% recombination; W1 to Ms1

with 24.3% ± 3% recombination. Gene order was proposed as Ms1 - Wm - W1 - Adh1 (Kiang, 1990).

Skorupska and Palmer (1990) showed the Beeson sterile (no Genetic Type number given) is allelic to the st5 st5 sterile and was linked to the W1 locus with percentage recombination of 24.7% ± 4.3%, giving another estimate of linkage between the St5 and W1 loci. Palmer et al.(1990) presented data showing several linkages: Y23 to W1 with 28.9% ± 1.1% recombination (F2 data); Y23 to Ms6 with 19.1% ± 2.0% recombination (F2:3 data); Y23 to St5 at 7.2% ± 1.7% recombination (F2:3 data).

Since Palmer and Kaul (1983) proposed gene order as Ms1 - breakpoint - W1 - Wm - St5, Palmer (1985) proposed gene order as Ms1 - Wm - W1 - St5, and Kiang (1990) proposed gene order as Ms1 - Wm - W1 - Adh1, we can expect gene order to be Ms1 - W1 - St5, Adh1 (omitting the translocation breakpoint and the Wm locus). Since Palmer et al. showed Y32 to be very close to St5, we can expect gene order to be Ms1 - W1 - St5, Y23, Adh1. Since Palmer et al. (1990) also showed percentage recombination between Y23 and Ms6 to be less than between Y23 and W1, we can expect gene order to be Ms1 - W1 - Ms6 - St5, Y23, Adh1. These are expectations only, and need confirmation.

The expectations of gene order are based on many different data sets of segregation of two loci each (two-point data). Linkage maps based on segregation of three or more loci would be preferred to determine order. Published linkage estimates have been calculated using the product method (Immer and Henderson, 1943), the maximum likelihood method (Allard, 1956), and the computer software program, LINKAGE-1 which is based on the maximum

likelihood method (Suiter et al., 1983). Estimates have been based on both F1:2 and F2:3 data. Published data involve many different genotypes and environments (locations and seasons). Recombination values can be influenced by the genotype and selfing environment (Mock, 1972; Pfeiffer and Vogt, 1989).

The purpose of this study is to provide additional three-point linkage data for six of the loci in Linkage Group 8 (all but Wm) using a common parent to produce F1 seed, and advancing the F1 seeds to the F2 in relatively similar environments. A summary linkage map will be made using percentage recombination values calculated from F1:2 data only.

MATERIALS AND METHODS

Ten lines were created which had the w1 allele and two other recessive alleles in coupling phase. Plants having a recessive allele at all three loci for a line were cross pollinated with cultivar BSR 101, having dominant alleles at all loci considered (Appendix A). Standard soybean cross-pollination techniques were used (Walker et al., 1979). All field studies were done either at the Bruner Farm near Ames, Iowa or at the University of Puerto Rico - Iowa State University Soybean Nursery at Isabela, Puerto Rico. All glasshouse studies were done either in the USDA glasshouse or in the Agronomy glasshouse at Iowa State University, Ames, Iowa. Percentage recombination values were calculated using the computer software program LINKAGE-1 (Suiter et al., 1983) for F1:2 data, or a modification of the product method (Allard, 1956) for F2:3 data. The procedures required to develop the genetic stocks for the experiments needed modification for each combination of three loci. Details of the procedures are described as ten separate experiments.

W1 Adh1 Ms1

Cultivar Wye, w1 w1 adh1 adh1 Ms1 Ms1 (Appendix A), was used as pod parent in cross pollinations with fertile plants from a white-flowered line, w1 w1 Adh1 Adh1 Ms1 --, derived from genetic type T266H, w1 w1 Adh1 Adh1 Ms1 ms1 (Palmer and Kiang, 1990). The F1 was advanced to the F1:2 in Puerto Rico. Selfed seeds from each pollen parent also were grown in Puerto Rico and scored for segregation of sterility; a segregating family

indicated a Ms1 ms1 heterozygous pollen parent. The F1:2 families from heterozygous pollen parents were grown at the Bruner Farm to determine which were segregating for sterility. Forty plants from each segregating F1:2 family were harvested individually. Fifty seeds from each F2:3 family were grown at the Bruner Farm to score for segregation of sterility.

Remnant F2:3 seeds from segregating families were planted at the Bruner Farm. Three seeds from each family were reserved for electrophoretic analysis to determine which families also were adh1 adh1 (Appendix A). Individual plants within adh1 adh1 families were given identification numbers. At flowering, pollen from these plants was examined microscopically to determine which plants were sterile. Pollen grains from ms1 ms1 plants are large and dark-staining (Albertsen and Palmer, 1979).

Cultivar BSR 101, W1 W1 Adh1 Adh1 Ms1 Ms1, was planted with the F2:3 families every second or third row. Sterile plants within adh1 adh1 families were used as pod parents in crosses with BSR 101. The goal of producing 30 F1 seeds was exceeded. The F1 seeds were advanced to the F1:2 in Puerto Rico.

Five seeds from each F1:2 family were tested electrophoretically (Appendix A) to confirm that the families were segregating for adh1. One hundred seeds from each of four F1:2 families, confirmed segregating for adh1, were germinated and sampled for electrophoretic analysis. The cotyledon samples from the seedlings were frozen at -86 °C for later evaluation. The seedlings were planted in peat pots. After several days

hardening off, the seedlings were transplanted to the field with their identities maintained.

The cotyledon samples were tested electrophoretically (Appendix A). At flowering, each transplant was scored for flower color, purple or white. At maturity, each transplant was scored as fertile or sterile by seed set and appearance. Plants were divided into eight phenotypic classes.

W1 Adh1 Ms6

Cultivar Wye is w1 w1 adh1 adh1 Ms6 Ms6 (Appendix A); genetic type T295H is w1 w1 Adh1 Adh1 Ms6 ms6 (Skorupska and Palmer, 1989). Wye was used as pollen parent in cross pollinations in Puerto Rico with ms6 ms6 plants. The F1 seeds were advanced to the F1:2 in Ames.

Fifty seeds from each F1:2 family were planted in Ames. Three remnant seeds from each family were tested electrophoretically to determine which families were adh1 adh1 (Appendix A). At flowering these families were scored in the field for segregation of sterility. Flowers of ms6 ms6 plants are small, and their anthers are translucent and have no pollen (Skorupska and Palmer, 1989). Sterile plants within adh1 adh1 families segregating for sterility were given an identification number.

Cultivar BSR 101, W1 W1 Adh1 Adh1 Ms6 Ms6, was planted with the F1:2 families every other row. Sterile plants within adh1 adh1 families were used as pod parents in crosses with BSR 101. The goal of producing 30 F1 seeds was not met. Twelve F1 seeds were advanced to the F1:2 in Puerto Rico. A total of 91 out-cross seeds from male-sterile plants within four adh1 adh1 families also were grown in Puerto Rico. At flowering, white-

flowered plants were removed. The remaining 12 purple-flowered plants were harvested; the expectation was that these plants were from out-crosses with BSR 101, since BSR 101 was planted on either side of the F1:2 families and was the only purple-flowered line near-by.

Fifteen seeds from the remnant seed of families used to cross with BSR 101 were tested electrophoretically to confirm that these families were adh1 adh1. A total of 800 seeds from 10 F1:2 families were germinated, and sampled for electrophoretic analysis; the cotyledon samples were frozen for later evaluation. The seedlings were planted in peat pots, and grown in a glasshouse. After several days hardening off, the seedlings were transplanted to the field with their identities maintained.

The cotyledon samples were tested electrophoretically (Appendix A). At flowering, each transplant was scored for flower color, purple or white. At maturity, each transplant was scored as fertile or sterile by seed set and appearance. Plants were divided into eight phenotypic classes.

W1 Adh1 St5

Cultivar Wye is w1 w1 adh1 adh1 St5 St5 (Appendix A); genetic type T272H is w1 w1 Adh1 Adh1 St5 st5 (Palmer and Kaul, 1983). Cross pollinations between Wye and fertile St5 -- plants were made. The F1 seeds were advanced to the F1:2 in Puerto Rico. The F1:2 families were grown in Ames and scored at maturity for segregation of sterility. Plants from each segregating family were harvested individually. Fifty seeds from each F2:3 family were grown in Ames to score for segregation of sterility.

Remnant F2:3 seeds from segregating families were planted at the Bruner Farm; three seeds from each family were reserved for electrophoretic analysis to determine which families also were adh1 adh1 (Appendix A). Individual plants within adh1 adh1 families were given an identification number.

Cultivar BSR 101, W1 W1 Adh1 Adh1 St5 St5, was planted with the F2:3 families every second or third row. At flowering, plants within adh1 adh1 families were scored for sterility using crossing glasses. Anthers of st5 st5 plants are shrunken, grey-yellow and shed little or no pollen. Fertile plants were used as pod parents in crosses with BSR 101. Our goal was to produce 90 F1 seeds, because only 2/3 of the fertile plants were expected to be St5 st5, and these would transmit the st5 allele only 1/2 the time. The goal of producing 90 F1 seeds was exceeded. The F1 seeds were advanced to the F1:2 in Puerto Rico.

Pod parents were harvested individually. Twenty-four selfed seeds from these plants were grown in Puerto Rico and scored for segregation of sterility to determine which of the pod parents were St5 St5 or St5 st5. Eighteen seeds from the pollen parents were tested electrophoretically to confirm that the pollen parents were adh1 adh1 (Appendix A). One parent was Adh1 Adh1, 13 parents were Adh1 adh1, and 1 parent was adh1 adh1.

Five F1:2 seeds from each w1 w1 Adh1 adh1 St5 st5 pod parent were tested electrophoretically to determine which F1:2 families were segregating for adh1. Fifty seeds from each of twenty F1:2 families segregating for adh1 were germinated and sampled for electrophoretic analysis. Cotyledon samples were frozen for later evaluation. The

seedlings were planted in peat pots, and grown in a glasshouse. After several days hardening off, the seedlings were transplanted to the field with their identities maintained (Appendix A).

At flowering, each of the 20 families was scored for segregation of sterility; all of the families were non-segregating. Crosses have been made to obtain a w1 w1 adh1 adh1 St5 st5 line, and crosses with BSR 101 will be repeated.

W1 Adh1 Y23

Cultivar Wye is w1 w1 adh1 adh1 Y23 Y23 (Appendix A); genetic type T288 is w1 w1 Adh1 Adh1 y23 y23 (Palmer et al., 1990). Cross pollinations, Wye x T288, were made; the F1 seeds were advanced to the F1:2. The F1:2 families were grown in Ames to score for segregation of chlorophyll-deficiency, and chlorophyll-deficient plants were harvested individually. Fifty F2:3 seeds were grown in Ames to confirm that each family was y23 y23.

Remnant F2:3 seeds were tested for alcohol dehydrogenase band 1 using starch gel electrophoresis, maintaining the identity of each individual (Appendix A). The seedlings were transferred to peat pots and grown in a glasshouse until they could be scored for chlorophyll-deficiency. Seedlings scored as adh1 adh1 null and as chlorophyll-deficient were hardened off for a few days and then transplanted to the field.

Cultivar BSR 101, W1 W1 Adh1 Adh1 Y23 Y23, was planted near the transplants, and used as pod parent in crosses. The goal of producing 30 F1 seeds was exceeded. The pollen parent identification number was

recorded for each cross. The F1 seeds were advanced to the F1:2 in Puerto Rico.

Pollen parents were harvested individually. Selfed seeds were tested electrophoretically to confirm that they were adh1 adh1. Every pollen parent was Adh1 Adh1. Crosses have been made to obtain a w1 w1 adh1 adh1 y23 y23 line, and crosses with BSR 101 will be repeated.

W1 Ms1 Y23

Genetic type T266H is w1 w1 Y23 Y23 Ms1 ms1; genetic type T288 is w1 w1 Ms1 Ms1 y23 y23. Cross pollinations between ms1 ms1 plants (pod parent) and T288 (pollen parent), were made. The F1 seeds were advanced to the F1:2 in Puerto Rico. F1:2 families were grown in Ames to insure that all families were segregating for both sterility and chlorophyll-deficiency; plants from each family were harvested individually. Fifty F2:3 seeds from each family were grown in Ames to determine which families were segregating for both sterility and chlorophyll-deficiency.

Remnant F2:3 seeds from these families were grown in the field at Ames. When the plants were clearly showing segregation for foliage color, chlorophyll-deficient plants were identified. At flowering, each chlorophyll-deficient plant was given an identification number, and pollen from these plants was examined microscopically to determine which were sterile. Pollen from ms1 ms1 plants is large and dark staining (Albertsen and Palmer, 1979).

Cultivar BSR 101, W1 W1 Ms1 Ms1 Y23 Y23 was planted with the F1:2 families every second or third row, and was used as pollen parent in

crosses with sterile chlorophyll-deficient plants. The goal of producing 30 F1 seeds was exceeded. The F1 seeds were advanced to the F1:2 in Puerto Rico.

All F1:2 seeds were hand planted at the Bruner Farm at the rate of four seeds per foot to avoid crowding. At flowering, individual plants within five F1:2 families with a total of 804 planted seeds were scored for flower color and foliage color. Also at flowering, anthers of small weak plants that were not expected to set pods regardless of genotype were examined with crossing glasses. Anthers of ms1 ms1 plants are large and shed little or no pollen. At maturity the plants were scored according to pod set as fertile with many pods or sterile with few or no pods. Plants were divided into eight phenotypic classes.

W1 Ms6 Y23

Genetic type T295H is w1 w1 Y23 Y23 Ms6 ms6 (Skorupska and Palmer, 1989); genetic type T288 is w1 w1 Ms6 Ms6 y23 y23 (Palmer et al., 1990). Cross pollinations between ms6 ms6 plants (pod parent) and T288 (pollen parent), were made. The F1 seeds were advanced to the F1:2 in Puerto Rico. F1:2 families were grown at the Bruner Farm to confirm that all families were segregating for both sterility and chlorophyll-deficiency; plants from each family were harvested individually. Fifty seeds from each F2:3 family were grown in Ames to determine which families were segregating for both sterility and chlorophyll-deficiency.

Remnant F2:3 seeds from these families were grown at the Bruner Farm. When the plants were clearly showing segregation for foliage color,

chlorophyll-deficient plants were identified. At flowering each chlorophyll-deficient plant was given an identification number. Anthers from these plants were examined microscopically to determine which were sterile. Anthers of ms6 ms6 plants have no pollen (Skorupska and Palmer, 1989).

Cultivar BSR 101, W1 W1 Ms6 Ms6 Y23 Y23 was planted with the F1:2 families every second or third row and was used as pollen parent in crosses with sterile chlorophyll-deficient plants. The goal of producing 30 F1 seeds was exceeded. The F1 seeds were advanced to the F1:2 in Puerto Rico.

All F1:2 seeds were hand planted at the Bruner Farm at the rate of four seeds per foot to avoid crowding. At flowering, individual plants within four F1:2 families with a total of 1174 planted seeds were scored for flower color and chlorophyll-deficiency. Also at flowering, flowers and anthers of small weak plants that were not expected to set pods regardless of genotype were examined with crossing glasses. Flowers of ms6 ms6 plants are small, and their anthers are translucent and shed no pollen (Skorupska and Palmer, 1989). At maturity the plants were scored according to pod set as fertile with many pods or sterile with few or no pods. Plants were divided into eight phenotypic classes.

W1 St5 Y23

Genetic type T288 is w1 w1 St5 St5 y23 y23 (Palmer et al., 1989); genetic type T272H is w1 w1 Y23 Y23 St5 st5. Cross pollinations between T288 (pod parent) and fertile T272H plants (pollen parent) were made. The F1 seeds were advanced to the F1:2 in Puerto Rico. F1:2 families were

grown at the Bruner Farm and scored for segregation for sterility and chlorophyll-deficiency. Plants from families segregating for both sterility and chlorophyll-deficiency were harvested individually. Fifty seeds from each F2:3 family were grown at the Bruner Farm to determine which were segregating for both sterility and chlorophyll-deficiency.

Remnant F2:3 seeds from these families were planted at the Bruner Farm. When the plants were clearly showing segregation for foliage color, chlorophyll-deficient plants were identified. At flowering, each chlorophyll-deficient plant was given an identification number, and pollen from these plants was examined microscopically to determine which were sterile. Pollen of st5 st5 plants is small, shrunken, and light-staining (Palmer and Kaul, 1983).

Cultivar BSR 101, W1 W1 St5 St5 Y23 Y23 was planted with the F1:2 families every second or third row and was used as pod parent in crosses with fertile chlorophyll-deficient plants. Our goal was to produce 90 F1 seeds, because only 2/3 of the yellow fertile plants were expected to be St5 st5, and these would transmit the st5 allele only 1/2 the time. The goal of producing 90 F1 seeds was exceeded. The F1 seeds were advanced to the F1:2 in Puerto Rico. Pollen parents were harvested individually. Twenty-four selfed seeds from these plants were grown in Puerto Rico to score for segregation of sterility to determine which of the pod parents were St5 st5. Germination and growth were poor; only four families were scorable, and these were non-segregating.

All F1:2 seeds were hand planted at the Bruner Farm at the rate of four seeds per foot to avoid crowding. At flowering, 17 plants within each

of 123 families were examined with crossing glasses in the field to find families segregating for sterility; only one family was found (A90-L-157). Individual plants were scored for flower color and foliage color. Also at flowering, anthers of small weak plants that were not expected to set pods regardless of genotype were examined with crossing glasses; anthers of st5 st5 plants are shrunken, grey-yellow and shed little or no pollen. At maturity, the plants were scored according to pod set as fertile with many pods or sterile with very few or no pods. Plants were divided into eight phenotypic classes.

Because we wanted more linkage data, we decided to generate additional F1:2 plants to evaluate. Two plants in this family were w1 w1 y23 y23 St5 --. These were used as pollen parents in crosses with BSR 101. The F1 seeds were advanced to the F1:2 in Puerto Rico. Selfed seeds from the two pollen parents were planted in the glasshouse to determine if the parents were St5 St5 or St5 st5. This progeny test was inconclusive due to the small number of seeds available. All F1:2 families will be planted at the Bruner Farm for evaluation in hopes that one or both parents were St5 st5.

Also because we wanted more linkage data, we decided to evaluate F2:3 families. All green, fertile, purple-flowered plants with at least 24 seeds were harvested from the family (A90-L-157). The seeds were planted in Puerto Rico. At flowering, each family was scored in the field for segregation of foliage color and flower color. Pollen was taken from plants in each family, and examined with a light microscope to score for segregation of sterility. Pollen of st5 st5 plants is small, shrunken, and light staining (Palmer and Kaul, 1983). At maturity, families were scored

again for segregation of sterility based on pod set and plant appearance. Families were divided into eight phenotypic classes.

W1 Ms1 St5

Genetic type T266H is w1 w1 St5 St5 Ms1 ms1 (Palmer and Kiang, 1990); Genetic type T272H is w1 w1 Ms1 Ms1 St5 st5 (Palmer and Kaul, 1983). Cross pollinations between ms1 ms1 plants (pod parent) and St5 -- plants from T272H (pollen parent) were made. The F1 seeds were advanced to the F1:2 in Puerto Rico. Crosses between fertile St5 -- plants from T272H (pod parent) and fertile Ms1 -- plants from T266H (pollen parent) also were made. The F1 seeds were advanced to the F1:2 at the Bruner Farm. F1:2 families were grown at the Bruner Farm and scored at maturity for segregation of sterility. Families segregating in a 3:1 fertile-to-sterile ratio were assumed segregating for only one type of sterility. Families segregating in a 9:7 fertile-to-sterile ratio were assumed segregating for both types of sterility. Plants from families segregating 9:7 were harvested individually. Fifty seeds from each F2:3 family were planted in Ames. Each family was scored at maturity according to pod set for segregation of each type of sterility. Sterile plants setting no pods were assumed to be st5 st5; sterile plants setting some pods were assumed to be ms1 ms1.

Remnant F2:3 seeds from families segregating for both types of sterility were planted at the Bruner Farm. At flowering, each plant was given an identification number. Pollen was collected from each plant and examined with a light microscope to identify ms1 ms1 plants. Pollen from ms1 ms1 plants is large and dark staining (Albertsen and Palmer, 1979).

Cultivar BSR 101 was planted with the F2:3 families every other row and used as pollen parent in crosses with ms1 ms1 plants in the F2:3 families. The goal was to generate 90 F1 seeds, because 2/3 of the ms1 ms1 plants setting pods were expected to be St5 st5, and these would transmit the st5 allele only 1/2 the time. The goal of producing 90 F1 seeds was exceeded. The F1 seeds were advanced to the F1:2 in Puerto Rico.

Every F1:2 family was planted at the Bruner Farm. At flowering, anthers were examined from 20 plants in each family to identify families segregating for sterility in a 9:7 ratio of fertile to sterile. Scoring was based on pollen shed and anther appearance. Three families segregating 9:7 with a total of 889 planted seeds were selected. Fertile purple-flowered plants within these families were removed and counted. Remaining non-flowering, white-flowered, or sterile plants were given identification numbers. Flower color also was recorded. Pollen was collected from each plant and examined with a light microscope. Pollen from ms1 ms1 plants is large and dark staining (Albertsen and Palmer, 1979). Pollen of st5 plants is small, shrunken, and light staining (Palmer and Kaul, 1983). Because we were not certain we could distinguish microscopically pollen of ms1 ms1 st5 st5 plants from pollen of ms1 ms1 St5 -- plants, or pollen of Ms1 -- st5 st5 plants, 20 suspected ms1 ms1 st5 st5 plants were examined in the field for pod set. We predicted they would express the st5 st5 phenotype and set very few or no pods. Palmer and Kaul (1983) observed only three seeds produced on 1000 st5 st5 plants. Plants were divided into eight phenotypic classes.

W1 Ms6 St5

Genetic type T295H is w1 w1 St5 St Ms6 ms6 (Skorupska and Palmer, 1989); Genetic type T272H is w1 w1 Ms6 Ms6 St5 st5 (Palmer and Kaul, 1983). Cross pollinations between ms6 ms6 plants (pod parent) and St5 -- plants (pollen parent) were made; the F1 seeds were advanced to the F1:2 in Puerto Rico or in Ames. F1:2 families were grown in Ames and scored at maturity for segregation of sterility. Families segregating in a 3:1 fertile-to-sterile ratio were assumed to be segregating for only one type of sterility. Families segregating in a 9:7 fertile-to-sterile ratio were assumed to be segregating for both types of sterility. Plants from families segregating 9:7 were harvested individually. Fifty seeds from each F2:3 family were planted at the Bruner Farm. Each family was scored at maturity according to pod set for segregation of each type of sterility. Sterile plants setting no pods were assumed to be st5 st5; sterile plants setting some pods were assumed to be ms6 ms6.

Remnant F2:3 seeds from families segregating for both types of sterility were planted at the Bruner Farm. At flowering, each plant was given an identification number. Pollen was collected from each plant and examined with a light microscope to identify ms6 ms6 plants. Anthers of ms6 ms6 plants have no pollen (Skorupska and Palmer, 1989).

Cultivar BSR 101 was planted with the F2:3 families every other row and used as pollen parent in crosses with ms6 ms6 plants in the F2:3 families. The goal was to generate 90 F1 seeds, because 2/3 of the ms6 ms6 plants setting pods were expected to be St5 st5, and these would transmit the st5 allele only 1/2 the time. The goal of producing 90 F1 seeds was

met. Most of the F1 seeds were advanced to the F1:2 in Puerto Rico. Poor quality F1 seeds were advanced to the F1:2 in the glasshouse in Ames.

Every F1:2 family was planted in the field at the Bruner Farm. At flowering, flowers and anthers from plants within each family were examined to find families segregating for both types of sterility. To quickly find ms6 ms6 sterility, white-flowered plants were examined; the w1 and ms6 loci are closely linked (Skorupska and Palmer, 1989). Flowers of ms6 ms6 plants are small, and their anthers are translucent and have no pollen (Skorupska and Palmer, 1989). Every family was segregating for ms6 sterility. To quickly find st5 sterility, purple-flowered plants were examined; because the w1 and ms6 loci are tightly linked (Skorupska and Palmer, 1989), and because ms6 seems to be epistatic over st5 at flowering, st5 st5 plants were more likely to be purple-flowered. Anthers of st5 st5 plants are shrunken, grey-yellow and shed little or no pollen (Palmer and Kaul, 1983). Four of 56 families, with a total of 364 planted seeds, were found. Fertile purple-flowered plants within these families were removed and counted. Anthers from remaining plants were examined, and plants were scored as fertile, ms6 ms6, or st5 st5. Flower color also was recorded. At maturity, pod set was recorded for all sterile plants. Plants scored ms6 ms6 at flowering with pod set at maturity were classified as ms6 ms6 St5 -- plants; those with no pod set at maturity were classified as ms6 ms6 st5 st5 plants. Plants were divided into eight phenotypic classes.

W1 Ms1 Ms6

Genetic type T266H is w1 w1 Ms6 Ms6 Ms1 ms1 (Palmer and Kiang, 1990); genetic type T295H is w1 w1 Ms1 Ms1 Ms6 ms6 (Skorupska and Palmer, 1989). Cross pollinations between Ms6 -- plants from T295H and Ms1 -- plants from T266H were made. The F1 seeds were advanced to the F1:2 in Ames. F1:2 families were grown in Ames and scored at maturity for segregation of sterility. Families segregating in a 3:1 fertile-to-sterile ratio were assumed segregating for only one type of sterility. Families segregating in a 9:7 fertile-to-sterile ratio were assumed segregating for both types of sterility. Plants from families segregating 9:7 were harvested individually. Fifty seeds from each F2:3 family were planted at the Bruner Farm. At maturity, each family was scored for segregation of each type of sterility according to fertile-to-sterile segregation ratios.

Remnant F2:3 seeds from families segregating for both types of sterility were planted at the Bruner Farm. At flowering, each plant was given an identification number. Pollen was collected from each plant and examined with a light microscope to identify ms6 ms6 plants. Anthers of ms6 ms6 plants have no pollen (Skorupska and Palmer, 1989).

Cultivar BSR 101 was planted with the F2:3 families every other row and used as pollen parent in crosses with ms6 ms6 plants in the F2:3 families. The goal was to generate 90 F1 seeds; 3/4 of the ms6 ms6 plants were expected to transmit the ms1 allele 2/3 of the time. The goal of producing 90 F1 seeds was exceeded. The F1 seeds were advanced to the F1:2 in Puerto Rico.

Every F1:2 family was planted at the Bruner Farm. At flowering, flowers and anthers from plants within each family were examined to find families segregating for both types of sterility. To quickly find ms6 ms6 sterility, white-flowered plants were examined; the w1 and ms6 loci are closely linked (Skorupska and Palmer, 1989). Flowers of ms6 ms6 plants are small, and their anthers are translucent and have no pollen (Skorupska and Palmer, 1989). Every family was segregating for ms6 sterility. To quickly find ms1 sterility, purple-flowered plants were examined; because w1 and ms6 are tightly linked (Skorupska and Palmer, 1989), and because ms6 ms6 seems to be epistatic over ms1 ms1 at flowering, ms1 ms1 plants were more likely purple-flowered. Anthers of ms1 ms1 plants are large and shed little or no pollen. Five families with a total of 846 planted seeds were selected. Fertile purple-flowered plants within these families were removed and counted. Anthers from remaining plants were examined and plants were scored as fertile, ms1 ms1 or ms6 ms6. Flower color also was recorded. We could not distinguish ms1 ms1 ms6 ms6 plants from Ms1 -- ms6 ms6 plants at flowering or at maturity. Plants were divided into six phenotypic classes. Eight phenotypic classes are required to calculate percentage recombination values.

We decided to evaluate F2:3 families to calculate percentage recombination values. Fertile plants with at least 48 seeds per plant were harvested from five families segregating in a 9:7 ratio. The F2:3 seeds were planted in Puerto Rico. At flowering, each family was scored for segregation of flower color. Pollen was taken from plants in each family, and examined with a light microscope to score for segregation for one or

both types of sterility. Families were divided into eight phenotypic classes.

RESULTS

F2 populations in each experiment except W1 Adh1 St5 and W1 Adh1 Y23 were scored for the phenotypes involved. Chi-square tests for segregation at each locus for each F1:2 family in each experiment were done using the software program LINKAGE-1 (Suiter et al., 1983). For some experiments, similar Chi-square calculations with F2:3 family data were made using the formula: Chi-square = Sum $[(|\#observed - \#expected| - 0.5)^2]/\#expected$. Chi-square values for each locus in each family are presented by experiment in Appendix B.

If the data for all loci in a family fit the expected ratio (5% probability level), the data for that family were used to determine recombination percentages. If the data for any locus in a family did not fit the expected ratio (5% probability), the data for that family were not used to determine recombination percentages. Recombination percentages are presented first by experiment, and second by loci pair across experiments. R ± SE is the recombination percentage plus or minus the standard error.

W1 Adh1 Ms1

Data from three F1:2 families with a total of 300 individuals were used to calculate percentage recombination values (Table 1). Based upon the summary percentage recombination values, the most likely gene order is Ms1 - W1 - Adh1 (Figure 1).

Table 1. Percentage recombination values for each loci pair in experiment W1 Adh1 Ms1 by family

Family Number	No. F2 Plants	Loci Pair and R ± SE[a]		
		W1 - Adh1	W1 - Ms1	Adh1 - Ms1
KA1	100	13.90 ± 5.01	44.91 ± 11.51	48.98 ± 12.10
KA3	100	16.30 ± 10.44	31.50 ± 9.58	33.68 ± 9.42
KA4	100	23.18 ± 10.34	24.16 ± 10.29	38.45 ± 9.28
Sum	300	17.95 ± 3.57	33.04 ± 5.56	41.25 ± 6.56

[a]R ± SE is percentage recombination plus or minus standard error.

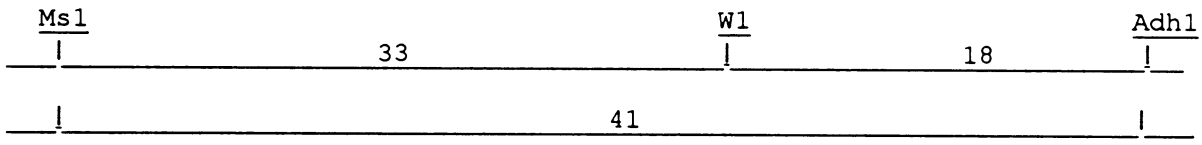

Figure 1. Linkage map based on summary values for experiment W1 Adh1 Ms1

W1 Adh1 Ms6

Data from eight F1:2 families with a total of 665 individuals were used to calculate percentage recombination values (Table 2). Based upon the summary percentage recombination values, the most likely gene order is W1 - Ms6 - Adh1 (Figure 2).

W1 Ms1 Y23

Data from five F1:2 families with a total of 683 individuals were used to calculate percentage recombination values (Table 3). Based upon the summary percentage recombination values, the most likely gene order is Ms1 - W1 - Y23 (Figure 3).

W1 Ms6 Y23

Data from four F1:2 families with a total of 1167 individuals were used to calculate percentage recombination values (Table 4). Based upon the summary percentage recombination values, the most likely gene order is W1 - Ms6 - Y23 (Figure 4).

W1 St5 Y23

Data from one F1:2 family with a total of 251 individuals were used to calculate percentage recombination values (Table 5). Data from 147 F2:3 families also were used to calculate percentage recombination values (Table 6). These calculations were made using a modification of the product method (Allard, 1956). A percentage recombination value could not be calculated using this method for the loci pair St5 - Y23, because there

Table 2. Percentage recombination values for each loci pair in experiment W1 Adh1 Ms6 by family

Family Number	No. F2 Plants	Loci Pair and R ± SE[a]		
		W1 - Adh1	W1 - Ms6	Adh1 - Ms6
KB1	80	25.52 ± 11.46	7.78 ± 12.31	19.01 ± 11.87
KB2	80	32.75 ± 10.44	6.30 ± 11.81	22.50 ± 11.16
KB3	80	13.19 ± 11.38	2.57 ± 11.62	13.19 ± 11.38
KB4	80	14.03 ± 11.58	0.00	14.03 ± 11.58
KB5	80	16.24 ± 11.41	4.58 ± 11.75	10.61 ± 11.62
KB6	80	28.61 ± 10.33	3.82 ± 11.38	23.72 ± 10.64
KB8	80	19.80 ± 11.23	4.22 ± 11.76	15.40 ± 11.45
KB10	105	18.88 ± 10.51	3.41 ± 10.96	14.96 ± 10.68
Sum	665	20.52 ± 2.63	3.96 ± 0.99	16.48 ± 2.27

[a]R ± SE is percentage recombination plus or minus standard error.

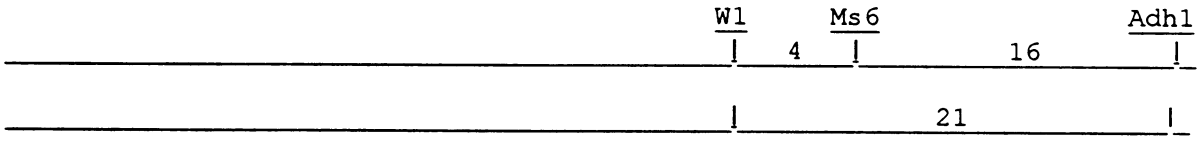

Figure 2. Linkage map based on summary values for experiment W1 Adh1 Ms6

Table 3. Percentage recombination values for each loci pair in experiment W1 Ms1 Y23 by family

Family Number	No. F2 Plants	Loci Pair and R ± SE[a]		
		W1 - Ms1	W1 - Y23	Ms1 - Y23
L-1	65	31.07 ± 11.05	15.21 ± 12.05	32.24 ± 10.96
L-2	121	31.11 ± 8.10	20.10 ± 8.65	41.44 ± 7.44
L-3	143	23.61 ± 5.88	19.85 ± 5.20	30.56 ± 7.12
L-4	243	31.13 ± 5.71	19.42 ± 6.13	41.30 ± 5.26
L-5	111	21.07 ± 8.99	19.03 ± 9.08	46.08 ± 7.42
Sum	683	28.04 ± 3.05	19.07 ± 2.31	38.80 ± 3.90

[a]R ± SE is percentage recombination plus or minus standard error.

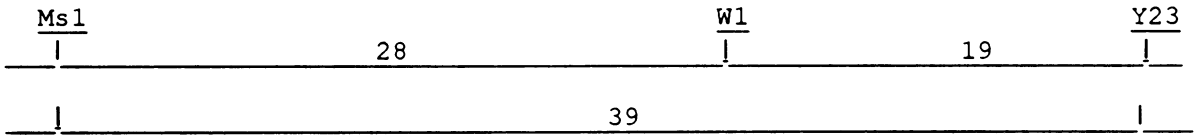

Figure 3. Linkage map based on summary values for experiment W1 Ms1 Y23

Table 4. Percentage recombination values for each loci pair in experiment W1 Ms6 Y23 by family

Family Number	No. F2 Plants	Loci Pair and R ± SE[a]		
		W1 - Ms6	W1 - Y23	Ms6 - Y23
L-32	368	3.45 ± 5.21	14.21 ± 5.08	12.14 ± 5.12
L-33	218	3.81 ± 6.78	17.97 ± 6.52	13.48 ± 6.62
L-34	296	3.52 ± 5.82	16.62 ± 5.63	13.46 ± 5.70
L-35	285	4.80 ± 5.92	19.78 ± 5.66	14.48 ± 5.77
Sum	1167	3.86 ± 0.69	16.89 ± 1.63	13.30 ± 1.40

[a]R ± SE is percentage recombination plus or minus standard error.

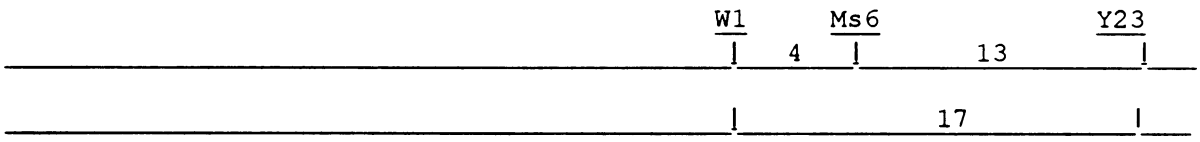

Figure 4. Linkage map based on summary values for experiment W1 Ms6 Y23

were no individuals in one of the eight phenotypic classes. Based upon the percentage recombination values from both the F2 and the F2:3 data, the most likely gene order is W1 - Y23 - St5 (Figures 5 and 6).

W1 Ms1 St5

Pollen of plants from two families was examined microscopically. Plants were divided into four classes based on pollen appearance (Figure 7). One class was normal pollen typical of fertile plants. One class was pollen typical of ms1 ms1 plants. One class was pollen typical of st5 st5 plants. Pollen of the fourth class was not uniform in grain size or degree of staining. Size and degree of staining ranged from as large and dark as pollen from ms1 ms1 plants to as small and light as pollen from st5 st5 plants. The majority of pollen was medium staining and sized between normal pollen and pollen from ms1 ms1 plants. The centers usually were shrunken away from the edges like pollen from st5 st5 plants. Twenty suspected ms1 ms1 st5 st5 plants examined in the field set no pods as predicted.

Data from two F1:2 families with a total of 649 individuals were used to calculate percentage recombination values (Table 7). Based upon the summary percentage recombination values, the most likely gene order is Ms1 - W1 - St5 (Figure 8).

Table 5. Recombination percentages for each loci pair in experiment W1 St5 Y23; F1:2 data

Family Number	No. F2 Plants	Loci Pair and R ± SE[a]		
		W1 - St5	W1 - Y23	St5 - Y23
L-157	251	18.52 ± 3.74	16.68 ± 3.49	2.37 ± 1.15

[a]R ± SE is percentage recombination plus or minus standard error.

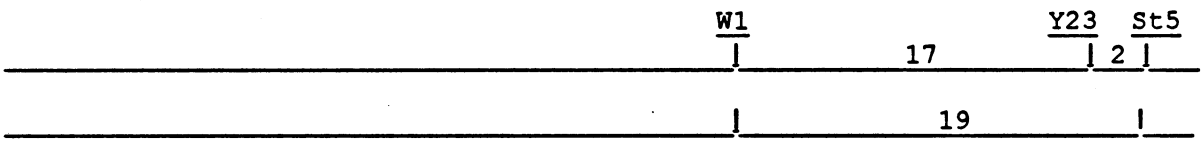

Figure 5. Linkage map based on F1:2 values for experiment W1 St5 Y23

Table 6. Percentage recombination values for each loci pair in experiment W1 St5 Y23; F2:3 data

No. of Families		Loci Pair and R ± SE[a]		
F2	F3	W1 - St5	W1 - Y23	St5 - Y23
1	147	17.70 ± 2.83	16.96 ± 2.72	undetermined

[a]R ± SE is percentage recombination plus or minus standard error.

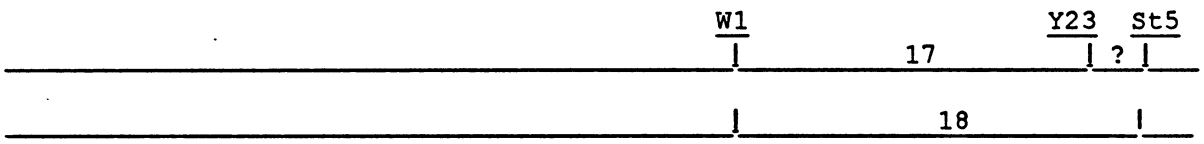

Figure 6. Linkage map based on F2:3 values for experiment W1 St5 Y23

Figure 7. Photomicrographs of pollen grains stained with iodine potassium iodide (750 x): A. Normal pollen grains from a fertile plant B. Pollen grains from a ms1 ms1 St5 -- plant C. Pollen grains from a Ms1 -- st5 st5 plant D. Pollen grains from a ms1 ms1 st5 st5 plant

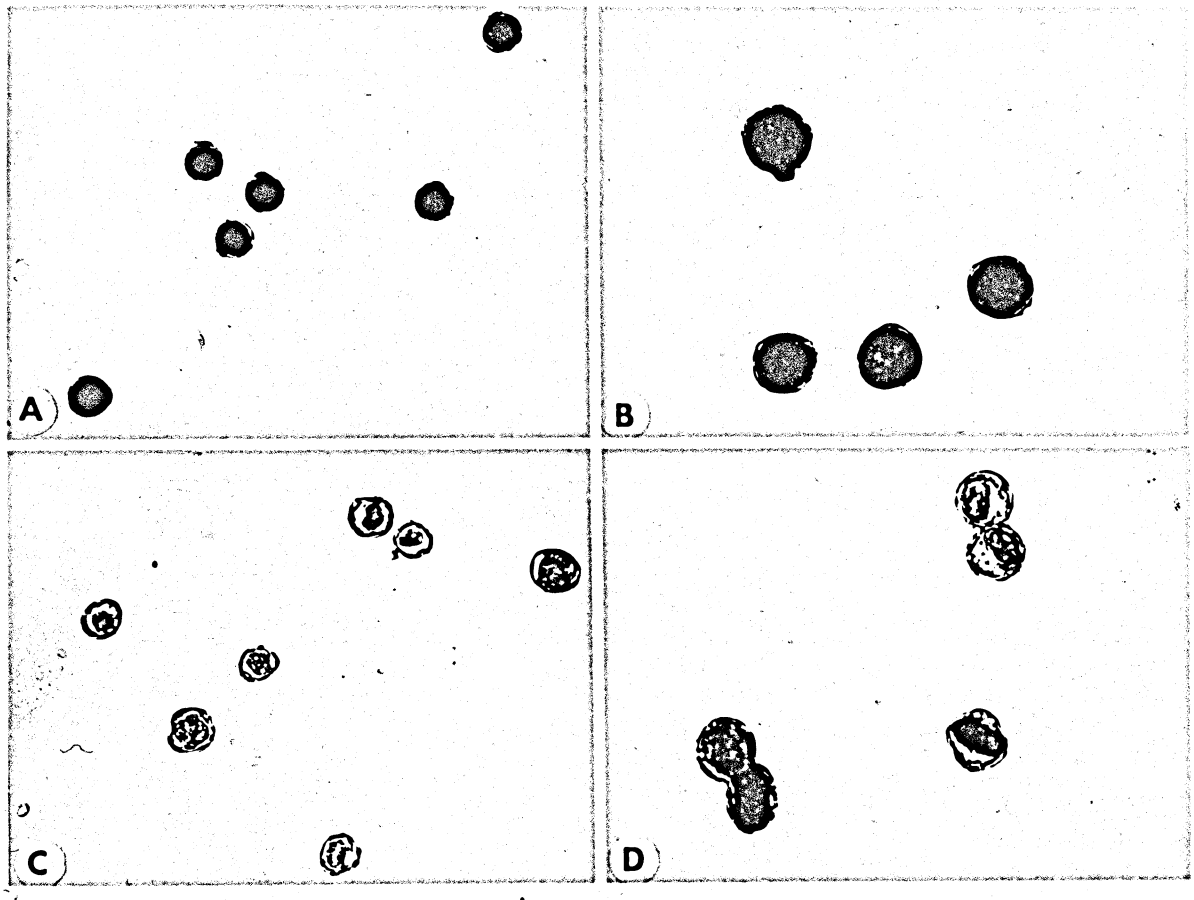
A
B
C
D

Table 7. Percentage recombination values for each loci pair in experiment W1 Ms1 St5 by family

Family Number	No. F2 Plants	Loci Pair and R ± SE[a]		
		W1 - Ms1	W1 - St5	Ms1 - St5
L-182	247	30.61 ± 5.71	15.60 ± 6.20	38.21 ± 5.38
L-183	402	30.79 ± 4.47	20.83 ± 4.75	38.76 ± 4.19
Sum	649	30.72 ± 3.37	18.94 ± 2.37	38.57 ± 4.00

[a]R ± SE is percentage recombination plus or minus standard error.

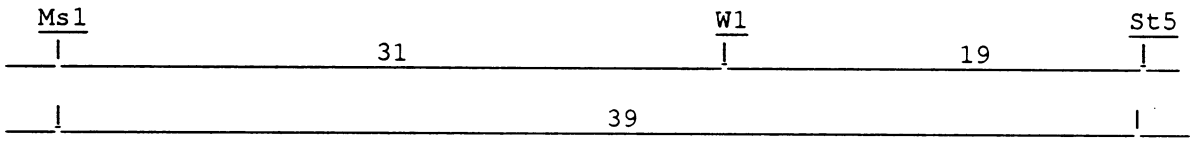

Figure 8. Linkage map based on summary values for experiment W1 Ms1 St5

W1 Ms6 St5

Data from three F1:2 families with a total of 329 individuals were used to calculate percentage recombination values (Table 8). To determine if pollen of ms6 ms6 st5 st5 plants could be distinguished microscopically from pollen of ms6 ms6 St5 -- plants, pollen was taken from 12 sterile plants in a F1:2 family segregating for ms6 and st5 sterility. Ten of the plants were scored ms6 ms6 and two of the plants were scored st5 st5 in the field. Pollen from these plants was examined microscopically. Pollen appeared as expected; there was no other phenotype indicating a ms6 ms6 st5 st5 plant. This indicates that the ms6 ms6 phenotype is epistatic over the st5 st5 phenotype at flowering as expected. Based upon the summary percentage recombination values, the most likely gene order is W1 - Ms6 - St5 (Figure 9).

W1 Ms1 Ms6

Data from 152 F2:3 families derived from five F2 families were used to calculate percentage recombination values (Table 9). To determine if pollen of ms1 ms1 ms6 ms6 plants could be distinguished microscopically from pollen of Ms1 -- ms6 ms6 plants, pollen was taken from 20 sterile plants in a family segregating for ms1 and ms6 sterility. Ten of the plants were scored ms1 ms1 and ten of the plants were scored ms6 ms6 in the field. Pollen from these plants was examined microscopically. Pollen appeared as expected; there was no other phenotype indicating a ms1 ms1 ms6 ms6 plant. This indicates that the ms6 ms6 phenotype is epistatic over the ms1 ms1 phenotype at flowering as expected.

Percentage recombination values were calculated using a modification of the product method (Allard, 1956). Based upon the summary percentage recombination values, the most likely gene order is place the Ms1 - W1 - Ms6 (Figure 10).

Loci Pair

Some loci pair were involved in more than one experiment. Summary percentage recombination values have been calculated for these loci pair using F2 data from all relevant experiments. F2:3 data were not used to calculate these summaries. Table 10 summarizes recombination values for all loci pairs for which F1:2 data were obtained.

Table 8. Percentage recombination values for each loci pair in experiment W1 Ms6 St5 by family

Family Number	No. F2 Plants	Loci Pair and R ± SE[a]		
		W1 - Ms6	W1 - St5	Ms6 - St5
L-225	84	3.67 ± 10.89	12.98 ± 10.69	8.80 ± 10.81
L-226	76	2.57 ± 11.46	17.38 ± 11.05	14.52 ± 11.18
L-246	169	6.52 ± 7.65	17.62 ± 7.40	10.49 ± 7.59
Sum	329	4.78 ± 1.46	16.36 ± 3.01	11.05 ± 2.35

[a]R ± SE is percentage recombination plus or minus standard error.

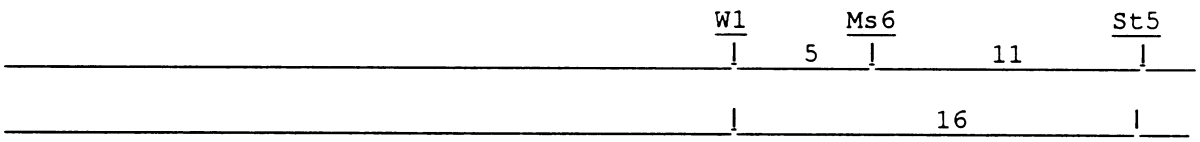

Figure 9. Linkage map based on summary values for experiment W1 Ms6 St5

Table 9. Percentage recombination values for each loci pair in experiment W1 Ms1 Ms6; F2:3 data

No. of Families		Loci Pair and R ± SE[a]		
F2	F3	W1 - Ms1	W1 - Ms6	Ms1 - Ms6
5	152	33.62 ± 5.84	2.49 ± 0.77	37.86 ± 6.68

[a]R ± SE is percentage recombination plus or minus standard error.

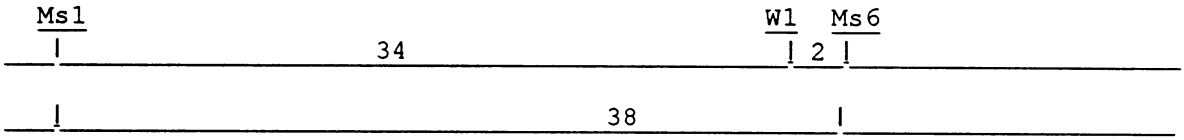

Figure 10. Linkage map based on summary values for experiment W1 Ms1 Ms6; F2:3 data

Table 10. Summary of percentage recombination values by loci pair across all F1:2 experiments

Loci Pair	Experiment	No. F1 Plants	No. F2 Plants	R ± SE[a]
W1 - Adh1	W1 Adh1 Ms1	3	300	17.95 ± 3.57
	W1 Adh1 Ms6	8	665	20.52 ± 2.63
	Sum	**11**	**965**	**19.59 ± 2.12**
W1 - Ms1	W1 Adh1 Ms1	3	300	33.04 ± 5.56
	W1 Ms1 Y23	5	683	28.04 ± 3.05
	W1 Ms1 St5	2	649	30.72 ± 3.37
	Sum	**10**	**1632**	**29.97 ± 2.10**
Adh1 - Ms1	W1 Adh1 Ms1	3	300	41.25 ± 6.56
W1 - Ms6	W1 Adh1 Ms6	8	665	3.96 ± 0.99
	W1 Ms6 Y23	4	1167	3.86 ± 0.69
	W1 Ms6 St5	3	329	4.78 ± 1.46
	Sum	**15**	**2161**	**4.04 ± 0.53**
Adh1 - Ms6	W1 Adh1 Ms6	8	665	16.48 ± 2.27
W1 - Y23	W1 Ms1 Y23	5	683	19.07 ± 2.31
	W1 Ms6 Y23	4	1167	16.89 ± 1.63
	W1 St5 Y23	1	251	16.69 ± 6.10
	Sum	**10**	**2101**	**17.55 ± 1.25**
Ms1 - Y23	W1 Ms1 Y23	5	683	38.80 ± 3.90
Ms6 - Y23	W1 Ms6 Y23	4	1167	13.30 ± 1.40
W1 - St5	W1 St5 Y23	1	251	18.53 ± 6.05
	W1 Ms1 St5	2	649	18.94 ± 2.37
	W1 Ms6 St5	3	329	16.36 ± 3.01
	Sum	**6**	**1229**	**18.21 ± 1.68**
St5 - Y23	W1 St5 Y23	1	251	2.37 ± 1.15
Ms1 - St5	W1 Ms1 St5	2	649	38.57 ± 4.00
Ms6 - St5	W1 Ms6 St5	3	329	11.05 ± 2.35

[a]R ± SE is percentage recombination plus or minus standard error.

DISCUSSION

The percentage recombination values of this study indicate a gene order that agrees with the expected gene order. Expected gene order was Ms1 - W1 - Ms6 - St5, Y23, Adh1. Our data give gene order as Ms1 - W1 - Ms6 - St5, Y23, Adh1. Due to overlapping standard errors, our data do not distinguish the order of St5, Y23, and Adh1 relative to W1 and Ms6. Crosses have been made to determine the order of St5, Y23, and Adh1 relative to W1 and Ms6.

Palmer (1985) tentatively placed the Wm locus between W1 and Ms1, stating gene order as Ms1 - Wm - W1 - St5. Kiang (1990) also placed the Wm locus between W1 and Ms1. Both publications involved several genotypes and F1:2 populations segregating for only two loci. The order of W1, Wm, and Ms6 is still uncertain. The Wm and Ms6 loci are closely linked to the W1 locus. Analysis of many F1:2 individuals from populations segregating at all three loci would provide additional linkage estimates that would be used to determine gene order.

The percentage recombination values of this study agree somewhat with published values. Some of our percentage recombination values are very close to published values, some are slightly different than published values, and some are considerably different than published values. Published linkage estimates for each loci pair and our linkage estimates for those loci pairs are presented together for comparison (Tables 11 - 18).

Three of our summary percentage recombination values are very close to published values. If standard errors are considered, the values overlap.

Table 11. Loci pair Adh1 - Ms1

Reference	Parents Used	Selfing Environment	No. F1 Plants	No. F2 Plants	C/R[a]	R ± SE[b]
Kiang, 1990	Jefferson, T267	glasshouse	not stated	762	R	42.1 ± 3
F1:2 Sum	BSR 101, Wye, T266H	Puerto Rico	3	300	C	41.25 ± 6.56

[a]Linkage phase in coupling (C) or repulsion (R).

[b]R ± SE is percentage recombination plus or minus standard error.

Table 12. Loci Pair W1 - Ms1

Reference	Parents Used	Selfing Environment	No. F1 Plants	No. F2 Plants	C/R[a]	R ± SE[b]
Kiang, 1990	Jefferson, T266	glasshouse	not stated	1476	R	24.3 ± 3
F1:2 Sum	BSR 101, Wye, T272H, T288, T266H	Puerto Rico	10	1632	C	29.97 ± 2.10
F2:3 data	BSR 101, T266H, T295H	Puerto Rico, then Ames, Iowa	5 F2 fam.	152 F2:3 fam.	C	33.62 ± 5.84

[a]Linkage phase in coupling (C) or repulsion (R).

[b]R ± SE is percentage recombination plus or minus standard error.

Table 13. Loci pair W1 - Adh1

Reference	Parents Used	Selfing Environment	No. F1 Plants	No. F2 Plants	C/R[a]	R ± SE[b]
Kiang and Chiang, 1987	Amsoy, AV68, Beeson, Ebony, A100, Hardee, Jefferson	glasshouse	not stated	1420	C	20.6 ± 1.2
Kiang, 1990	Jefferson, T266	glasshouse	not stated	1476	C	24.9 ± 0.9
Sum	BSR 101, Wye, T295H, T266H	Puerto Rico	11	965	C	19.59 ± 2.12

[a]Linkage phase in coupling (C) or repulsion (R).

[b]R ± SE is percentage recombination plus or minus standard error.

Table 14. Loci pair W1 - Ms6

Reference	Parents Used	Selfing Environment	No. F1 Plants	No. F2 Plants	C/R[a]	R ± SE[b]
Skorupska and Palmer, 1989	T295, Calland, Cutler, Hark	Puerto Rico	not stated	3854	C	3.08 ± 0.1
Sum	BSR 101, Wye, T295H, T272H, T288	Puerto Rico	11	2161	C	4.04 ± 0.53

[a]Linkage phase in coupling (C) or repulsion (R).

[b]R ± SE is percentage recombination plus or minus standard error.

Table 15. Loci pair W1 - St5

Reference	Parents Used	Selfing Environment	No. F1 Plants	No. F2 Plants	C/R[a]	R ± SE[b]
Palmer and Kaul, 1983	T272H, Clark, PI84,896	Puerto Rico	not stated	1738	C	26.7 ± 1.9
Skorupska and Palmer, 1990	Beeson-sterile, T272H	Puerto Rico	not stated	not stated	R	24.7 ± 4.3
Sum	BSR 101, T295H T272H, T288, T266H	Puerto Rico	6	1229	C	18.21 ± 1.68

[a]Linkage phase in coupling (C) or repulsion (R).

[b]R ± SE is percentage recombination plus or minus standard error.

Table 16. Loci pair W1 - Y23

Reference	Parents Used	Selfing Environment	No. F1 Plants	No. F2 Plants	C/R[a]	R ± SE[b]
Palmer et al., 1990	T288, trisomic D, Hark-fr1	Ames, Iowa	not stated	2600	C	28.9 ± 1.1
Sum	BSR 101, T266H, T295H, T272H, T288	Puerto Rico	10	2101	C	17.55 ± 1.25

[a]Linkage phase in coupling (C) or repulsion (R).

[b]R ± SE is percentage recombination plus or minus standard error.

Table 17. Loci pair Ms6 - Y23

Reference	Parents Used	Selfing Environment	No. F1 Plants	No. F2 Plants	C/R[a]	R ± SE[b]
Palmer et al., 1990	T288, T295H	Ames, Iowa	not stated	286 F2:3 fam.	C	19.1 ± 2.0
Sum	BSR 101, T295H, T288	Puerto Rico	4	1167	C	13.30 ± 1.40

[a]Linkage phase in coupling (C) or repulsion (R).

[b]R ± SE is percentage recombination plus or minus standard error.

Table 18. Loci pair St5 - Y23

Reference	Parents Used	Selfing Environment	No. F1 Plants	No. F2 Plants	C/R[a]	R ± SE[b]
Palmer et al.,	T272H, T288	Ames, Iowa	not stated	79 F2:3 fam.	C	7.2 ± 1.7
Sum	BSR 101, T272H, T288,	Puerto Rico	1	251	C	2.37 ± 1.15

[a]Linkage phase in coupling (C) or repulsion (R).

[b]R ± SE is percentage recombination plus or minus standard error.

Our summary percentage recombination value for loci pair Adh1 - Ms1 agrees with that of Kiang (1990)(Table 11.). Our summary value for loci pair W1 - Ms1 agrees with the 30.3% ± 0.9% value published by Palmer (1985)(data was not presented), yet is somewhat larger than that of Kiang (1990)(Table 12). Our summary value for loci pair W1 - Adh1 agrees with that of Kiang and Chiang (1987), yet is somewhat smaller than that of Kiang (1990)(Table 13).

Two of our summary percentage recombination values are somewhat close to published values, but when standard errors are considered, they do not overlap. Our summary percentage recombination value for loci pair W1 - Ms6 is somewhat larger than that of Skorupska and Palmer (1989)(Table 14). Our summary value for loci pair W1 - St5 is somewhat smaller than that of Palmer and Kaul (1983) and that of Skorupska and Palmer (1990)(Table 15).

Our summary recombination values for the loci pairs mentioned so far agree with or are relatively close to published values. However, our summary recombination values for loci pairs W1 - Y23, Ms6 - Y23, and St5 - Y23 are all much lower than published values (Tables 16, 17, and 18). Published values for these loci pairs all came from one study, involving the y23 y23 mutant, in which the selfing environment was Ames, Iowa. (Palmer et al., 1990). We cannot explain the differences between our recombination values and published recombination values for loci pairs W1 - Y23, Ms6 - Y23, and St5 - Y23.

Palmer et al. (1990) used F2:3 data to calculate recombination values for loci pairs Ms6 - Y23 and St5 - Y23. It is more time-consuming to score as many F2:3 families as F1:2 plants, so F2:3 data typically are based on fewer F2 individuals. In this example, 286 F2:3 families vs. 1167 F1:2

plants for loci pair Ms6 - Y23 (Table 17), and 79 F2:3 families vs. 251 F1:2 plants for loci pair St5 - Y23 (Table 18) were used to calculate recombination values. However, to calculate a recombination value for loci pair W1 - Y23, Palmer et al. (1990) used data from 2600 F1:2 plants vs. the data from 2101 F1:2 plants used in our study (Table 16).

Recombination values can be influenced by several factors in addition to experimental error. Mock (1972) summarized both intrinsic and extrinsic factors that can influence recombination in animals and plants. These included genotype, environment (especially temperature and soil fertility), genotype by environment interaction, which parent was maternal or paternal, linkage phase, and other factors. Mock (1972) cited several examples of temperature affecting recombination values. It is possible that environmental temperature during meiosis may influence percentage recombination in soybean.

There is some evidence that genotype affects recombination in soybean. Kiang (1987) reported greater percentage recombination for the loci pair Ap - Lap1 using data from a G. max x G. max population (23.6% ± 1.0%) than from a G. soja x G. soja population (19.9% ± 1.1%). Griffin and Palmer (1987b) calculated two very different percentage recombination values for the loci pair Sp1 - Aco3. A value of 4.6% ± 0.9% was calculated using F1:2 data from an interspecific cross, Glycine max x G. soja. A value of 30.65 ± 3.0% was calculated using F1:2 data from a G. max x G. max population. Griffin and Palmer (1987b) concluded that cryptic structural differences between the G.max and G.soja accessions used affected recombination.

Pfeiffer and Vogt (1989), using soybean, investigated the effect of environment on recombination values. They reported percentage recombination values for loci pair Ln - P2 that ranged from 24.1% ± 1.5% to 38.9% ± 2. They presented recombination estimates across 11 year-by-location environments. An analysis of variance indicated significant differences among environments (year-by-location), but no significant differences among the three locations involved.

Percentage recombination estimates for a loci pair can vary greatly. Griffin et al. (1989) reported a summary percentage recombination value of 41.4% ± 0.8% for loci pair Fr1 - Ep. They reported no difference in estimate of recombination between reciprocal crosses or between linkage phase. Data were based on a diverse selection of genotypes and environments, yet were homogeneous by a Chi-square test (P = 0.43). However, the range was from 33.9% ± 2.9% to 47.8% ± 3.3%. Griffin and Palmer (1987a) reported a percentage recombination value of 30.8% ± 1.6% for loci pair Sp1 - T. They reported a range of 26.5% ± 2.9% to 37.5% ± 3.7%, yet reported the data homogenous by a Chi-square test (P = 0.15). Hildebrand et al. (1980) first reported a percentage recombination value of 16.2% ± 1.5% for loci pair Ap - T1. Kiang (1987), using different cultivars than Hildebrand et al. (1980), reported a percentage recombination value of 6.6% ± 0.5% for this loci pair. Because of the apparent discrepancy, Yu and Kiang (1990) used the same cultivars used by Hildebrand et al. (1980) to re-estimate the recombination value for loci pair Ap - T1. Yu and Kiang (1990) obtained a percentage recombination value of 6.1% ± 0.8%.

In view of the ranges of percentage recombination values reported by other investigators, the percentage recombination values obtained in this study are not very different from those of published studies. On the contrary, some are remarkably similar. Further studies with Linkage Group 8 are already in progress, and will provide additional percentage recombination values to compare with our values and published values.

Linkage Group 8 will be studied not just to resolve any discrepancies between recombination values, but because of the unusual nature of the linkage group and its chromosome. Linkage Group 8 was assigned to the satellite chromosome or nucleolus organizer chromosome by Sadanaga and Grindeland (1984) using Trisomic S which has three satellite chromosomes. The nucleolus organizer chromosome of any species also is associated with ribosomal RNA synthesis. Skorupska et al. (1989) detected rRNA coding genes on the satellite chromosome using in situ hybridization with a biotinylated maize rRNA probe. The satellite chromosome has been designated chromosome 13 by Singh and Hymowitz (1988). They also provided the first karyotype of soybean including measurements of the chromosomes. They reported that chromosome 13 has the greatest long-arm/short-arm length ratio and is only 16.8% heterochromatic; yet, the short arm is entirely heterochromatic. Heterochromatin is transcriptionally inactive, late replicating, and is associated with chromosome breaks. Also, three of the six interchanges or translocations characterized in soybean are associated with the satellite chromosome (Palmer and Kilen, 1987; Sellner, 1990). The satellite chromosome has been of great interest in cytogenetic studies.

In addition, the Ms1 locus on the satellite chromosome has been useful to cytogenetic studies. The ms1 ms1 mutant is interesting, because sterility is caused by failure of post-meiotic cytokinesis, which is unusual (Albertsen and Palmer, 1979; Hymowitz et al., 1991). Another unusual characteristic of the Ms1 locus is that there have been seven independent mutations at this locus (Skorupska and Palmer, 1990). Seeds from ms1 ms1 plants are also a source of triploids and other polyploids, aneuploids from triploids, and haploids, all of which are useful in cytogenetic studies including mapping linkage groups to chromosomes (Hymowitz et al., 1991). Linkage Group 8 is unusual and deserves further investigation.

CONTINUING RESEARCH

To determine gene order of loci Adh1, Y23 and St5 in relation to W1, we have decided to generate F1:2 data involving these four loci in coupling phase. Cross pollinations have been made to create a white-flowered, w1 w1, line segregating for the adh1,y23, and st5 alleles. Seeds from this line will be sown on germination paper and sampled for electrophoretic analysis (Appendix A). Seedlings will be transplanted to peat pots and grown in the glasshouse. Seedlings scored adh1 adh1 by electrophoretic analysis will be scored for chlorophyll-deficiency. Hypocotyl color should be green for all seedlings. After hardening off, seedlings scored w1 w1 adh1 adh1 y23 y23 will be transplanted to the field at the Bruner Farm near Ames, Iowa. Fertile plants, St5 St5 or St5 --, from this line will be used as pollen parents in cross pollinations with plants of 'BSR 101'. F1 seeds will be advanced to the F1:2 at the University of Puerto Rico - Iowa State University soybean nursery at Isabela, Puerto Rico.

Pollen parents will be progeny tested to determine which were St5 st5. Progeny testing will be done in an environment that allows differentiation between a sterile chlorophyll-deficient plant and a weak, fertile, chlorophyll-deficient plant. Remnant seed from w1 w1 adh1 adh1 y23 y23 St5 st5 pollen parents will stored as genetic maintenance stock.

F1:2 seeds from families from heterozygous pollen parents (St5 st5) will be sown on germination paper and sampled for electrophoretic analysis. Seedlings will be transferred to peat pots; after hardening off, they will be transplanted to the Bruner Farm. At flowering, transplants will be scored for flower color and plant color. At maturity, transplants will be

scored fertile or sterile based on pod set and plant appearance. Percentage recombination values will be calculated using the computer software program LINKAGE-1 which utilizes the maximum likelihood method (Suiter et al., 1983).

We would like to have two additional lines for genetic maintenance stocks: w1 w1 adh1 adh1 y23 y23 Ms1 --, and w1 w1 adh1 adh1 y23 y23 Ms6 --. Cross pollinations have been made to create a white-flowered (w1 w1) line segregating for the adh1, y23, and ms1 alleles; and a white-flowered (w1 w1) line segregating for the adh1, y23, and ms6 alleles. Seeds from these lines will be sown on germination paper and sampled for electrophoretic analysis. Seedlings will be transplanted to peat pots and grown in the glasshouse. Seedlings scored adh1 adh1 by electrophoretic analysis will be scored for chlorophyll deficiency. Hypocotyl color should be green for all seedlings. After hardening off, seedlings scored w1 w1 adh1 adh1 y23 y23 will be transplanted to the field near Ames. At maturity, transplants will be scored fertile or sterile based on pod set and plant appearance. Fertile plants will be harvested and progeny tested to determine which are heterozygous for male-sterility. Remnant seed from plants heterozygous for male-sterility will be stored as genetic maintenance stocks. These lines would be available to use in further linkage studies.

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APPENDIX A: A STARCH GEL ELECTROPHORETIC PROCEDURE FOR RESOLVING SOYBEAN ALCOHOL DEHYDROGENASE BAND 1

Introduction

Electrophoretic analysis of plant isozyme polymorphisms has become an important tool for plant geneticists and evolutionists (Weeden and Wendel, 1989). The techniques used are dependent upon the species and enzymes studied (Kephart, 1990; Wendel and Weeden, 1989) Electrophoretic techniques for soybean include polyacrylamide gel electrophoresis (PAGE), starch gel electrophoresis, and polyacrylamide/starch gel electrophoresis (Hedges et al., in press).

Gorman and Kiang (1977) used PAGE to identify three zymogram patterns for soybean alcohol dehydrogenase (ADH, EC 1.2.3.4). Pattern 1 had a seven-band zymogram; pattern 2 had a five-band zymogram with bands 1 and 4 missing; pattern 3 had a four-band zymogram with bands 1, 4 and 5 missing. Gorman and Kiang (1978) suggested a model of inheritance for ADH bands. They postulated that bands 3, 6 and 7 are related, and that bands 1, 4 and 5 also are related; ..."bands 1 and 5 being the respective homodimers of loci designated ADH-1 and ADH-4 and band 4 being the heterodimer formed by the combination of monomers from ADH-1 and ADH-4." The gene symbol later was changed to Adh1. Kiang and Chiang (1987), using polyacrylamide/starch gel electrophoresis, reported genetic linkage of the Adh1 locus and the W1 locus in Linkage Group 8.

Rennie et al. (1989) used starch gel electrophoresis for soybean ADH and found only two distinct invariant bands. In a 1989 personal communication to R.G. Palmer, Charlie Pedersen described a procedure using

starch gel electrophoresis that consistently differentiated between soybean ADH patterns 1 and 3 but not pattern 2; Gorman and Kiang's (1977) band 1 was not resolved. Using a morpholine-citrate buffer system (pH 8.3), Delorme and Skorupska (1992) used starch gel electrophoresis for soybean ADH. They resolved four strong bands and two weak bands. They reported that their soybean ADH banding patterns did not correlate with those reported by Gorman and Kiang (1978).

The analysis of soybean ADH using PAGE and polyacrylamide/starch electrophoresis has the advantage of resolving more bands more consistently than starch gel electrophoresis, but has the disadvantage of being more expensive and involving hazardous chemicals. It would be desirable if the safer and less expensive starch gel electrophoresis procedure could be used to reliably resolve all seven bands described by Gorman and Kiang (1977). This would be especially desirable for use in further studies of Linkage Group 8, since band 1, the band which has been difficult to resolve using starch gel electrophoresis, is the putative homodimer of the locus designated Adh1 by Gorman and Kiang (1978).

This paper describes a starch gel electrophoretic procedure which resolves all seven soybean ADH bands. The methods used are based on those described by Cardy and Beversdorf (1984) and Rennie et al. (1989). Several modifications were made at different stages of the procedure.

Sample Preparation

The most significant modification is to sample the cotyledons 6 h to 24 h after planting on germination paper. This is far earlier than the 96

h to 240 h recommended by Rennie et al. (1989). The intensity of band 1 is greatest when cotyledons are sampled at 6 h and is only slightly less intense when sampled at 12 h, 18 h, or 24 h. When cotyledons are sampled at 30 h, resolution of band 1 is not reliable; band 1 does not resolve when cotyledons are sampled at 36 h, 42 h, or 48 h. The seed coats are removed before sampling; this is facilitated by wetting the seed coats with distilled water. A core sample is taken through both cotyledons. Care is taken to not break off either cotyledon or damage the embryo. The young seedlings are easily transplanted to peat pots.

Samples are placed in microcentrifuge tubes with 30 uL of homogenization buffer, (16.7 g sucrose and 8.3 g sodium ascorbate added to 100 mL distilled deionized water). Tubes with reusable lids are useful if the sample is processed in stages or has to be re-run. To maintain high enzyme quality, samples are stored in an ultra-low freezer at -86 °C.

Samples are ground for 45 sec with the sample tube immersed in a small jar of ice. The sample is centrifuged for 1.5 min at 12,400 rpm in a Fisher Microcentrifuge, model 235B. A pre-punched wick (Northfork Products, P.O. Box 4347, Tumwater, WA. 98501 USA) is placed into each sample to absorb supernatant before re-freezing.

Buffer and Gel Preparation

Decreasing the amount of citric acid in the electrode buffer from 1.5 g/L to 0.55 g/L increases the pH from 6.5 to 6.8 and increases separation and resolution of ADH. Buffer freshness is extremely important; use of week-old buffer results in poor resolution of band 1.

Gels are prepared with 500 mL of gel buffer (1 part electrode buffer: 3 parts distilled deionized water) and either 65 g (13% w/v), 60 g (12% w/v) or 55 g (11% w/v) of starch. Higher starch concentration improves band clarity of the stained gel slices, which is desirable if the slices are to be photographed. The starch is suspended in 150 mL of gel buffer; 350 mL of gel buffer is cooked in a microwave on high for 220 sec before adding to the starch suspension and cooking on high for approximately 40 to 45 sec. After being wrapped in Saran Wrap and cooled to room temperature overnight, the gels are refrigerated approximately 15 min before loading with samples.

Electrophoresis and Staining

Gels are run 5.5 h. A pair of gels usually is run using one power source at 16 W constant wattage; a single gel is run using a power source at 8 W. After electrophoresis, a double-thickness bottom slice is used for staining to ease handling and to increase the intensity of band 1. The staining solution is a modification of that in Rennie et al. (1989); 0.15 g agar is dissolved in 10 mL 0.1 M Tris-HCl (pH 8.0) in a microwave oven on high 35 sec to 40 sec then added to a premixed solution of 10 mL 0.1 M Tris-HCl (pH 8.0), 2 mL (25 mg/50 mL) NAD, 2 mL (25 mg/50 mL) NBT, 0.5 mL (10 mg/50 mL) PMS, and 5 drops of 95 % ethanol. The mixture is poured over the gel slice and allowed to set before incubating in the dark at 38 °C. While bands 2 through 7 appear within 2 h, band 1 may not appear for 6 h, so scoring is done the next morning.

Evaluation of Banding Patterns

Eight cultivars have been evaluated using this procedure: Beeson, BSR 101, Cayuga, Cutler, Jefferson, Lincoln, Swift, and Wye. We observed three banding patterns, similar to those illustrated by Gorman and Kiang (1977), among the eight cultivars. A seven-band pattern is observed for Beeson, BSR 101, Cutler and Swift. A five-band pattern is observed for Lincoln and Wye. A four-band pattern is observed for Cayuga and Jefferson (Figure 1).

There is some unexplained non-genetic variability between runs; band 1 and band 4 appear in different positions relative to the other bands. Relative-position differences are associated with the citric acid content of the electrode buffer, and the wattage used during electrophoresis. Use of higher citric acid content and higher wattage results in band 1 being closer to band 2 and in band 4 being closer to band 5.

Discussion

The observed banding pattern types for Beeson, Cayuga, Cutler, Jefferson and Lincoln are the same as those reported by Gorman et al. (1982), while the banding pattern types observed for Swift and Wye are different than reported by Gorman et al. (1982). They reported a type 2 banding pattern (five bands) for Swift and a type 3 banding pattern (four bands) for Wye.

The banding patterns observed using this procedure and those described and illustrated by Gorman and Kiang (1977) are similar enough to allow the use of this safer, less expensive procedure to differentiate between soybean ADH patterns 1, 2 and 3, and to score for Adh1 in future

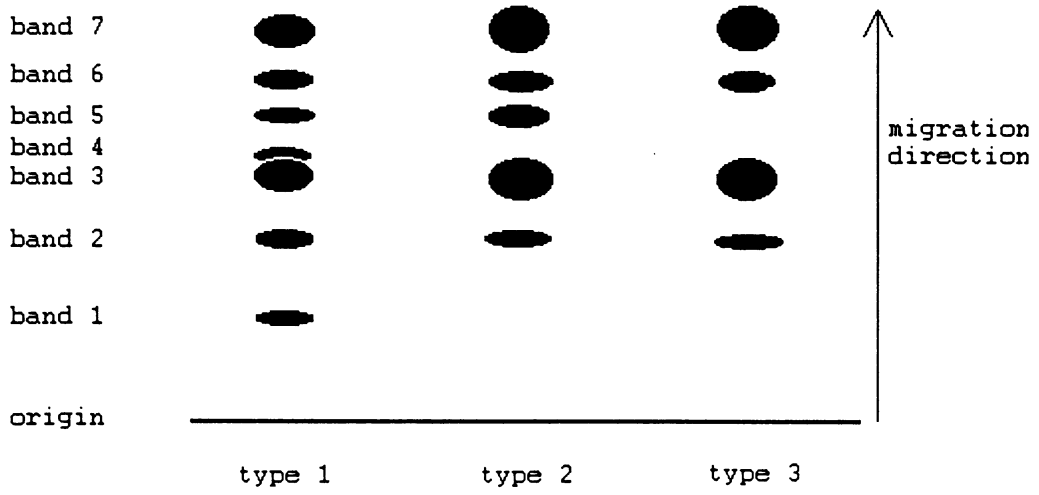

Figure 1. Observed ADH banding patterns in soybean

genetic linkage studies with Linkage Group 8. However, the unexplained non-genetic variability between runs should be investigated. Human ADH is a dimeric zinc metaloenzyme (Jornvall et al., 1987). It is possible soybean ADH also may be a metaloenzyme. Citric acid, a component of the electrode buffer, is a strong chelator of divalent metal ions. The citric acid could interact with a divalent ion associated with the enzyme and alter its electrophoretic mobility. Further study of soybean ADH structure and inheritance is required.

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APPENDIX B: CHI-SQUARE VALUES

F1:2 populations were scored for the phenotypes involved. Chi-square tests for segregation at each locus for each family in each experiment were done using the software program LINKAGE-1 (Suiter et al.,1983). For some experiments, similar Chi-square calculations with F2:3 family data were made using the formula: Chi-square = Sum [(|#observed - #expected| - $0.5)^2$]/#expected. If the data for each locus in a family fit the expected 3:1 ratio (5% probability level), the data for that family were used to determine summary Chi-square values for the loci in that experiment. If the data for any locus in a family did not fit the expected 3:1 ratio (5% probability), the data for that family were not used. Chi-square values for each locus in each family are presented by experiment.

W1 Adh1 Ms1

Four hundred F2 seedlings were sampled for electrophoretic analysis and transplanted to the field. Transplants were scored for flower color during flowering and for fertility/sterility at maturation. Only data for the Adh1 locus were obtained from seedlings which died before being scored for flower color or sterility. The number of plants with dominant phenotypes, the number of plants with recessive phenotypes, and Chi-square values are included in Table 1. Family KA2 was not included in the summary Chi-square calculations because of high Chi-square values at the W1 and Adh1 loci. Data from families KA1, KA3, and KA4 were used to obtain summary Chi-square values and recombination percentages.

Table 1. Chi-square values by family for each locus in experiment W1 Adh1 Ms1

Family Number	No. F2 Plants	W1 Locus		Adh1 Locus		Ms1 Locus	
		Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq
KA1	100	75:21	0.50	76:24	0.05	70:26	0.22
KA2	100	60:33	5.45*	64:36	6.45*	62:31	3.44
KA3	100	66:20	0.14	79:21	0.85	66:20	0.14
KA4	100	54:28	3.66	68:32	2.61	61:21	0.02
Sum	300	195:69	0.18	223:77	0.07	197:67	0.02

[a]Expected ratio 3:1, Dom:Rec.

*Significant at the .05 probability level.

W1 Adh1 Ms6

Eight hundred F2 seedlings were sampled for electrophoretic analysis and transplanted to the field. Transplants were scored for flower color during flowering and for fertility/sterility at maturation. Only data for the Adh1 locus were obtained from seedlings which died before being scored for flower color or sterility. The number of plants with dominant phenotypes, the number of plants with recessive phenotypes, and Chi-square values are included in Table 2. Data from family KB7 were not included in the summary Chi-square calculations because of a high Chi-square value at the Adh1 locus. Data from family KB9 were not included in Table 2, because family KB9 did not segregate at the Adh1 locus. Data from families KB1, KB2, KB3, KB4, KB5, KB6, KB8, and KB10 were used to obtain summary Chi-square values and recombination percentages.

W1 Ms1 Y23

Five F2 families with a total of 683 plants were scored. The number of plants with dominant phenotypes, the number of plants with recessive phenotypes, and Chi-square values are included in Table 3. Data from all five families were used to obtain summary Chi-square values and recombination percentages.

W1 Ms6 Y23

Four F2 families with a total of 1167 plants were scored. The number of plants with dominant phenotypes, the number of plants with recessive phenotypes, and Chi-square values are included in Table 4. Data from all

Table 2. Chi-square values by family for each locus in experiment W1 Adh1 Ms6

Family Number	No. F2 Plants	W1 Locus		Adh1 Locus		Ms6 Locus	
		Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq
KB1	80	49:16	0.01	59:21	0.07	46:19	0.62
KB2	80	58:13	1.69	65:15	1.67	56:15	0.57
KB3	80	52:22	0.88	54:26	2.40	52:22	0.88
KB4	80	54:17	0.04	63:17	0.60	54:17	0.04
KB5	80	59:13	1.85	62:18	0.27	56:16	0.30
KB6	80	55:22	0.52	63:17	0.60	56:21	0.21
KB7	30	16:8	0.89	17:13	5.38*	15:9	2.00
KB8	80	52:20	0.30	62:18	0.27	55:17	0.07
KB10	105	57:26	1.77	79:26	0.00	58:25	1.16
Sum	665	436:149	0.07	507:158	0.55	433:152	0.30

[a]Expected ratio 3:1, Dom:Rec.

*Significant at the .05 probability level.

Table 3. Chi-square values by family for each locus in experiment W1 Ms1 Y23

Family Number	No. F2 Plants	W1 Locus		Ms1 Locus		Y23 Locus	
		Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq
L-1	65	44:21	1.85	50:15	0.13	46:19	0.62
L-2	121	89:32	0.13	88:33	0.33	98:23	2.32
L-3	143	117:26	3.55	112:31	0.84	114:29	1.70
L-4	243	189:54	1.00	175:68	1.15	186:57	0.31
L-5	111	86:25	0.36	86:25	0.36	88:23	1.08
Sum	683	525:158	1.27	511:172	0.01	532:151	3.05

[a]Expected ratio 3:1, Dom:Rec.

Table 4. Chi-square values by family for each locus in experiment W1 Ms6 Y23

Family Number	No. F2 Plants	W1 Locus		Ms6 Locus		Y23 Locus	
		Dom:Reca	Chi-sq	Dom:Rec a	Chi-sq	Dom:Rec a	Chi-sq
L-32	368	287:81	1.75	285:83	1.17	289:79	2.45
L-33	218	164:53	0.04	166:52	0.15	165:53	0.06
L-34	296	224:71	0.14	225:69	0.37	227:69	0.45
L-35	285	218:66	0.47	218:67	0.34	212:73	0.06
Sum	1167	893:271	1.44	894:271	1.88	893:274	1.43

[a]Expected ratio 3:1, Dom:Rec.

four families were used to obtain summary Chi-square values and recombination percentages.

W1 St5 Y23

One F2 family with a total of 251 plants was scored. The number of plants with dominant phenotypes, the number of plants with recessive phenotypes, and Chi-square values are included in Table 5. Data from this family were used to calculate recombination percentages.

One-hundred-forty-seven W1 - - St5 - - Y23 - - F1:2 plants were threshed individually. The F2:3 seeds were planted in Puerto Rico. At flowering each family was scored for segregation of foliage color, flower color, and sterility. The expected ratio of non-segregating families to segregating families at each locus was 1:2. The number of families not segregating, the number of families segregating, and Chi-square values at each locus are presented in Table 6. Chi-square calculations were made using the formula: Chi-square = Sum [(|#observed - #expected| - $0.5)^2$]/#expected. Linkage calculations were made based on these data.

W1 Ms1 St5

Two F2 families with a total of 649 plants were scored. The number of plants with dominant phenotypes, the number of plants with recessive phenotypes, and Chi-square values are included in Table 7. Some plants could not be scored for all three loci. Data from both families were used to obtain summary Chi-square values and recombination values.

Table 5. Chi-square values for each locus in experiment W1 St5 Y23; F1:2 data

Family Number	No. F2 Plants	W1 Locus		St5 Locus		Y23 Locus	
		Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq
L-157	251	179:72	1.82	185:66	0.22	185:66	0.22

[a]Expected ratio 3:1, Dom:Rec.

Table 6. Chi-square values for each locus in experiment W1 St5 Y23; F2:3 data

No. F2:3 Fam.	W1 Locus		St5 Locus		Y23 Locus	
	NS:Sega	Chi-sq	NS:Sega	Chi-sq	NS:Sega	Chi-sq
147	58:89	2.45	49:98	0.00	48:99	0.01

[a]Expected ratio 1:2, NS:Seg.

Table 7. Chi-square values by family for each locus in experiment W1 Ms1 St5

Family Number	No. F2 Plants	W1 Locus		Ms1 Locus		St5 Locus	
		Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq
L-182	247	187:60	0.07	190:55	0.85	191:54	1.14
L-183	402	287:114	2.51	301:99	0.01	285:115	3.00
Sum	649	474:174	1.19	491:154	0.43	476:169	0.50

[a]Expected ratio 3:1, Dom:Rec.

W1 Ms6 St5

Four F2 families with a total of 341 plants were scored. The number of plants with dominant phenotypes, the number of plants with recessive phenotypes, and Chi-square values are included in Table 8. Family L-439 was generated in the glasshouse in Ames. Families L-225, L-226, and L-246 were generated in Puerto Rico; data from these three families were used to obtain summary Chi-square values and recombination values.

W1 Ms1 Ms6

One-hundred-fifty-two W1 - - Ms1 - - Ms6 - - plants from five F1:2 families segregating at all three loci were threshed individually. The F2:3 seeds were planted in Puerto Rico. At flowering each family was scored for segregation of flower color, ms1 ms1 sterility, and ms6 ms6 sterility. The expected ratio of non-segregating families to segregating families at each locus was 1:2. The number of families not segregating, the number of families segregating, and Chi-square values at each locus are presented in Table 9. Chi-square calculations were made using the formula: Chi-square = Sum $[(|\#\text{observed} - \#\text{expected}| - 0.5)^2]/\#\text{expected}$. Linkage calculations were made based on these data.

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Table 8. Chi-square values by family for each locus in experiment W1 Ms6 St5

Family Number	No. F2 Plants	W1 Locus		Ms6 Locus		St5 Locus	
		Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq	Dom:Rec[a]	Chi-sq
L-225	84	64:20	0.06	63:21	0.00	64:20	0.06
L-226	76	55:21	0.28	55:21	0.28	52:24	1.75
L-246	169	133:36	1.23	135:34	2.15	129:40	0.16
L-439	12	7:5	1.78	7:5	1.78	7:5	1.78
Sum	329	252:77	0.45	253:76	0.63	245:84	0.05

[a]Expected ratio 3:1, Dom:Rec.

Table 9. Chi-square values for each locus in experiment W1 Ms1 Ms6; F2:3 data

F2 Family Number	No. F2:3 Fam	W1 Locus		Ms1 Locus		Ms6 Locus	
		NS:Seg[a]	Chi-sq	NS:Seg[a]	Chi-sq	NS:Seg[a]	Chi-sq
L-287	29	14:15	2.45	10:19	0.00	12:17	0.34
L-288	31	10:21	0.00	9:22	0.04	10:21	0.00
L-304	43	14:29	0.00	16:27	0.24	12:31	0.24
L-315	16	6:10	0.07	4:12	0.07	6:10	0.07
L-316	33	9:24	0.31	10:23	0.03	7:26	1.67
Sum	152	53:99	0.07	49:103	0.07	48:104	0.18

[a]Expected ratio 1:2, NS:Seg.